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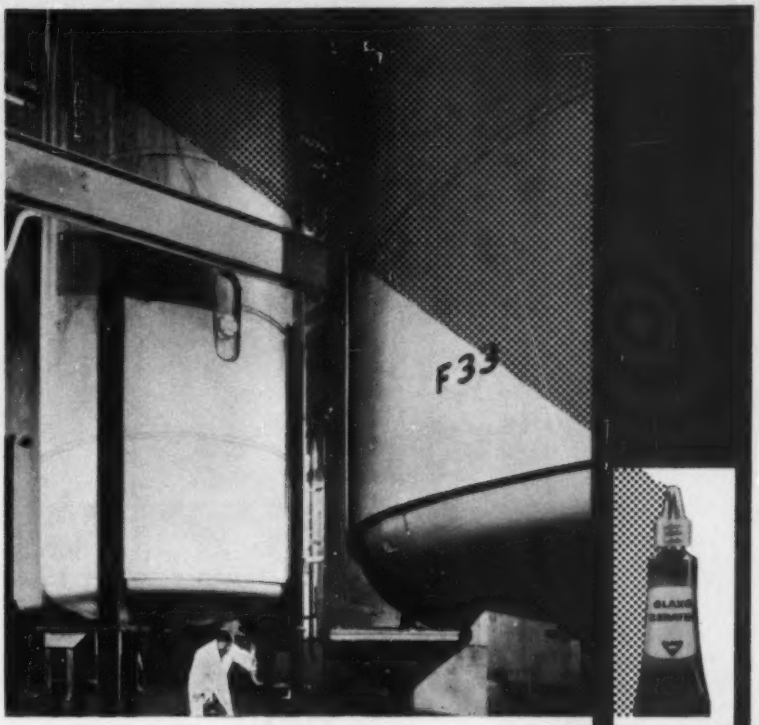

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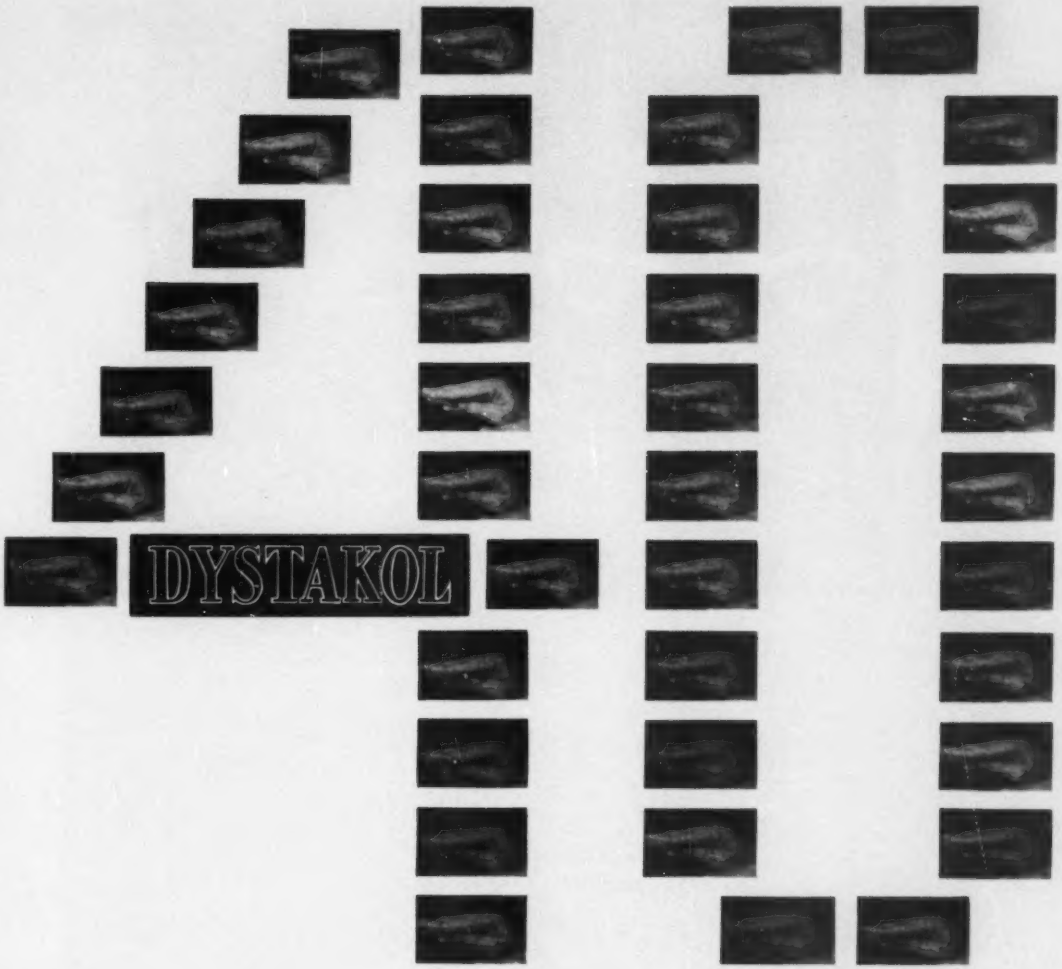

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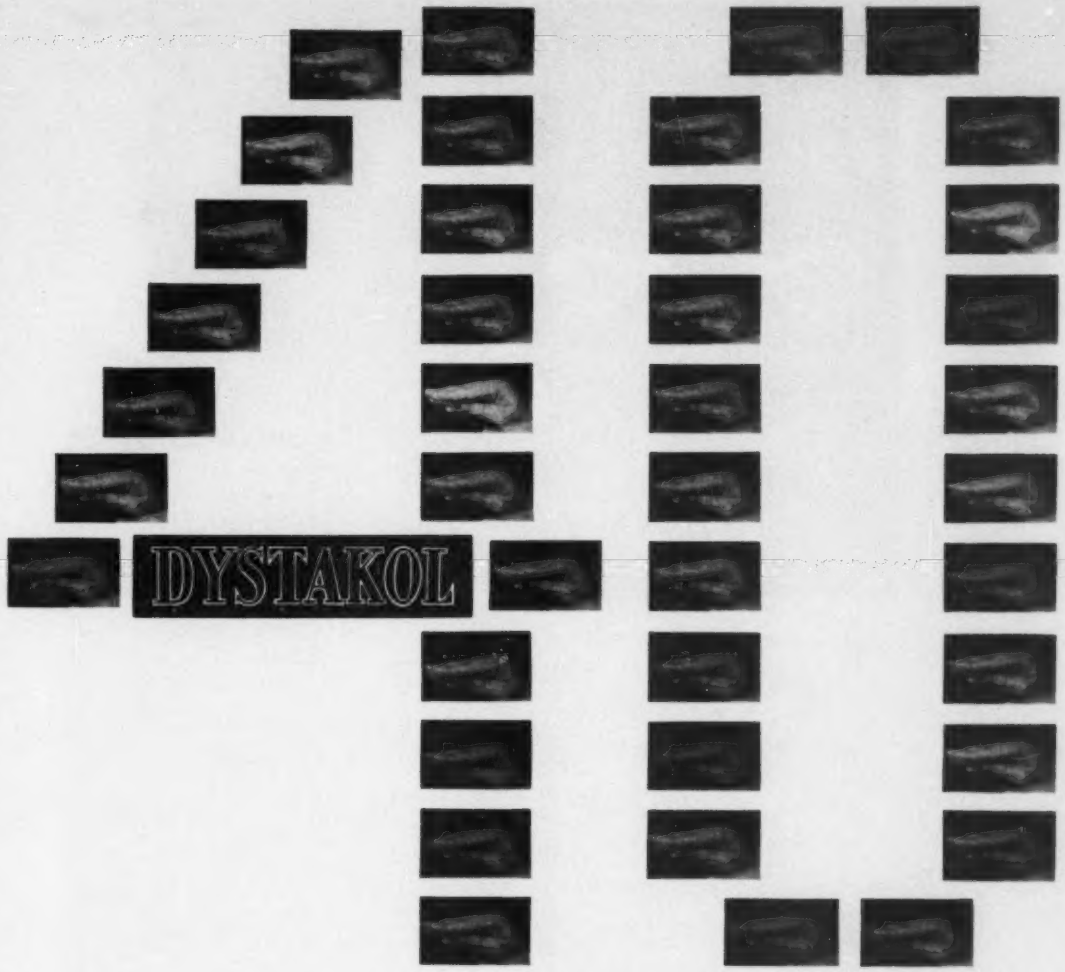

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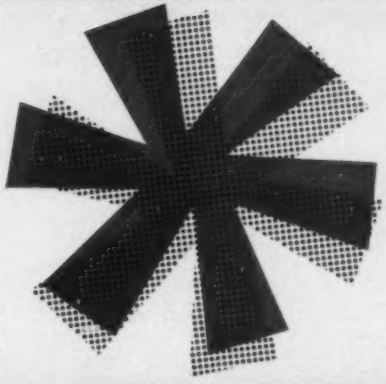

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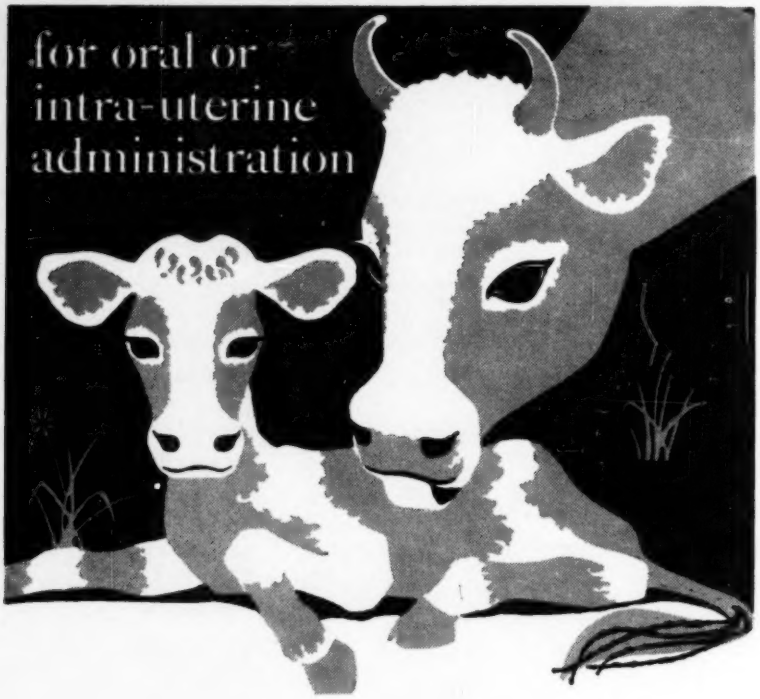

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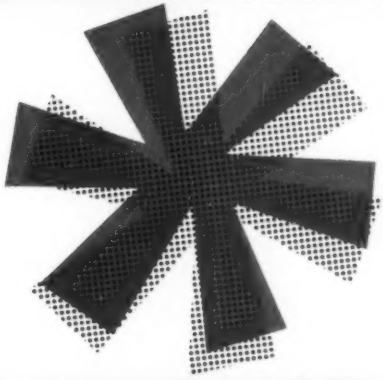

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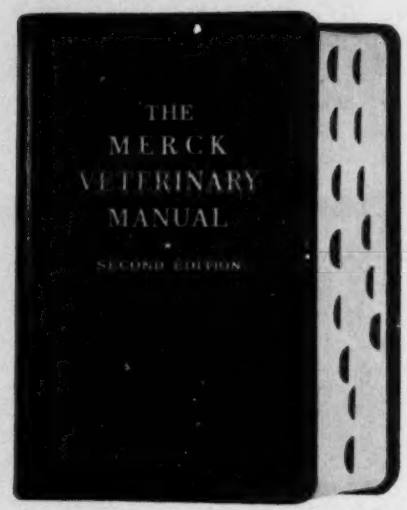

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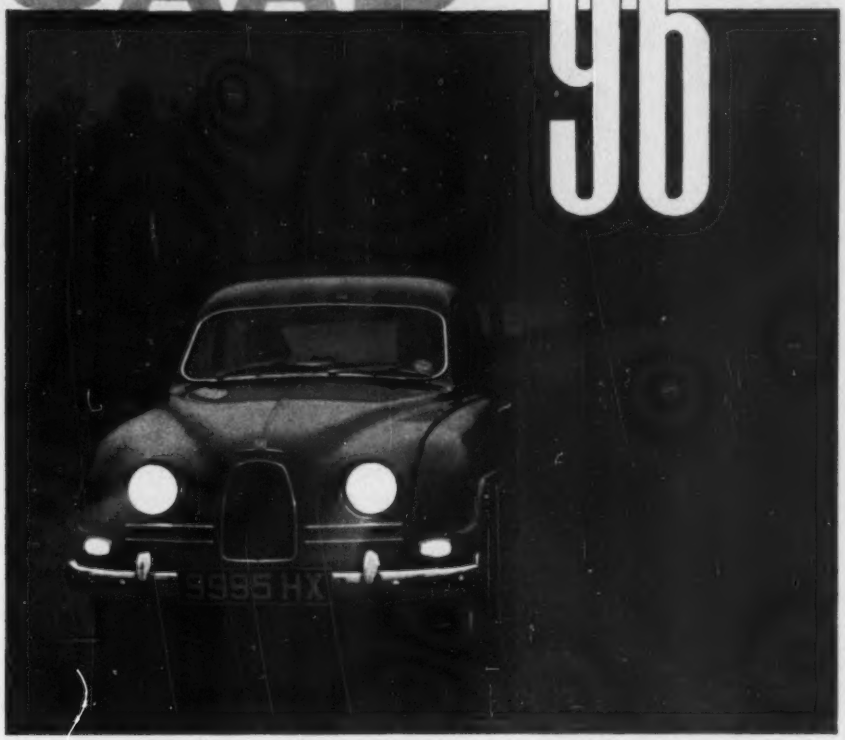

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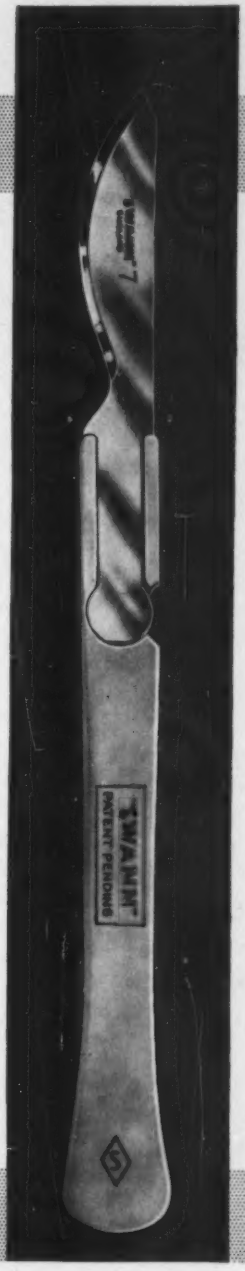

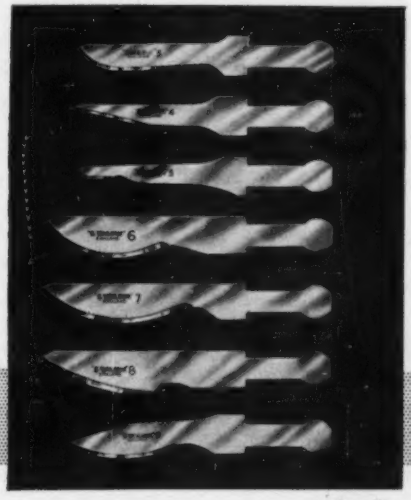

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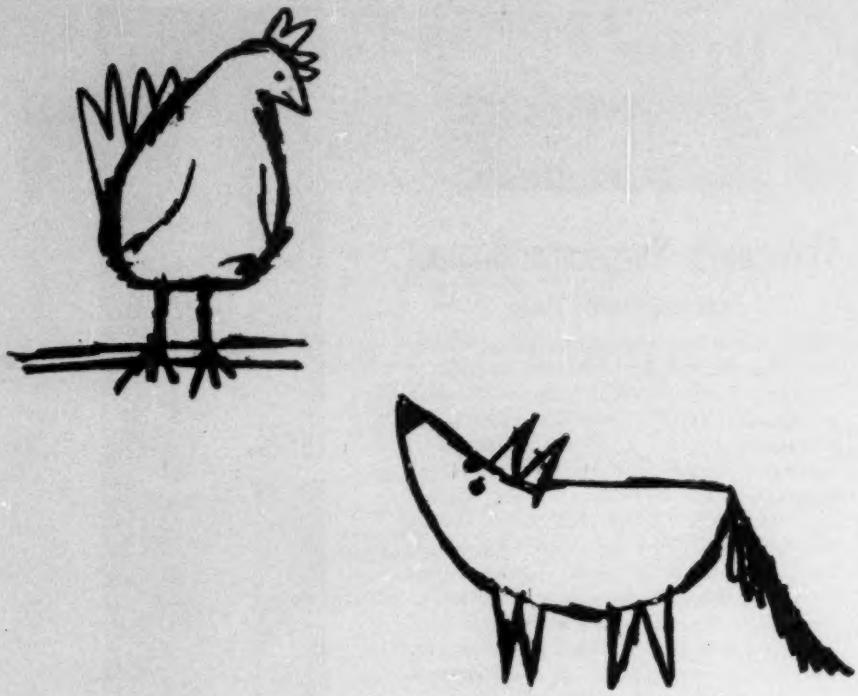

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THE CELLULAR PICTURE OF VAGINAL MUCUS FROM NORMAL HEIFERS

By I. D. B. Newsam*

Veterinary Investigation Centre, Ministry of Agriculture, Fisheries and Food, Cambridge

The cellular picture of bovine vaginal mucus has been studied extensively but there is a measure of disagreement on the cell content, and its variation, in the normal animal. Frei & Metzger (1926) reported cornified elements as being the outstanding feature of smears prepared at oestrus; Zupp (1926) and Schneerson (1936) agreed. But Hammond (1927) found that oestrus mucus was characterized by the presence of a few epithelial cells, and Cole (1930) considered that a large epithelial cell predominated at all stages of the cycle. Hammond reported that polymorphonuclear leucocytes were a feature of smears prepared up to the sixth day after oestrus and thereafter only occasional cells of this series could be seen, but Frei and Metzger found large numbers of leucocytes from the tenth to the 15th day.

The work now reported was undertaken to gain more definite information on the cell content of vaginal mucus from normal, virgin, heifers, and to determine how the cell picture altered during the oestrus cycle.

MATERIAL AND METHODS

Twelve Jersey heifers, approximately 15 months old, were purchased from a tuberculin-tested herd known to be free from brucellosis. The heifers had been reared on a farm where there were no bulls, and were free from venereally transmitted infectious diseases. Blood calcium, magnesium, copper and inorganic phosphorus levels of the animals were within the normal range. Before the experiment began the heifers were observed through a total of 89 complete oestrus cycles; the average duration was 20·5 days and the range 16 to 24 days. It was considered that the heifers were healthy, normal virgin stock. They were housed throughout the experiment and received a concentrate and hay ration sufficient to keep them in good bodily condition.

Mucus samples were aspirated from the anterior vagina with sterile glass pipettes 45 cm. long, of 7 mm. internal bore diameter. The 12 animals were each sampled daily for at least one complete oestrus cycle and in all 16 cycles were examined in detail. As far as possible, samples were collected at the same time each day to allow a full 24 hours between samples. To eliminate the possibility that daily sampling might cause a cellular response due to minor mechanical trauma, the group of animals was used as a whole to provide the picture for one oestrus cycle; no animal was sampled more than twice in this

* Present address: C.S.I.R.O., Private Bag 1, Parkville, N.2, Victoria, Australia.

period and those animals used on two occasions had a rest period of at least seven days between samplings.

Smears of the vaginal mucus were made within 30 minutes after collection. Pledgets of non-absorbent cotton wool were rotated in the mucus sample whilst it was still retained in the pipette. The swab so prepared was then rolled over the surface of clean glass slides and the preparation fixed immediately in 50 per cent ether/absolute alcohol for 15 minutes. Preparations were stained according to the method of Shorr (1941), as modified by Foot (1945), using Revector Brand dyes (Hopkins & Williams Ltd., Chadwell Heath, Essex). Smears were examined with a two-thirds objective and the components given a rating varying from ++++ when present in innumerable numbers (cells) or excessive quantity (mucus), to ± when cells could only be found after searching many fields. The percentage number of monocyte cells was calculated by counting 400 cells of the leucocyte series.

RESULTS

Examination of the smears showed that variation in the cellular content of vaginal mucus occurred during the normal oestrus cycle, and although the picture was never exactly similar in any two cycles the following data illustrate the types of cells present and the changes seen. Regular daily sampling of the experimental animals by the method detailed did not influence the cell content of the mucus.

Cell Content of Vaginal Mucus from Non-pregnant Virgin Heifers

Epithelial cells, polymorphonuclear leucocytes, monocytes, eosinophils and occasional lymphocytes could be distinguished clearly. The limiting membrane of both cell and nucleus of the leucocyte series was usually well defined, the cytoplasm faint blue and the nucleus red to purple in colour. Red-staining, intracytoplasmic granules could often be seen scattered uniformly throughout the cytoplasm. Monocytes varied in number from nil to 5 per cent of the total leucocyte count; eosinophils appeared only when erythrocytes could be demonstrated; lymphocytes were rare (Fig. 1).

The vaginal epithelial cell was oval or circular and varied in diameter from 15 μ to 30 μ or more. The limiting membrane, in carefully prepared smears, was intact. The cytoplasm stained a faint blue, and red granular inclusions were a variable feature. The nucleus was central in position, occupied about one-third of the cell and stained red to purple; its limiting membrane was well defined and the chromatin network and granules were evident (Figs. 2, 3 and 4).

A feature of the epithelial cells was the demonstration, within them, of engulfed leucocytes (Figs. 2 and 3) and also what appeared to be plasma cells (Fig. 4). Generally the included cells were single but occasionally they were so numerous as to occlude the cytoplasm and displace the central nucleus to the periphery of the cell. In many cases the engulfed cell was surrounded by a halo, giving the impression that it was within a vacuole (Fig. 2). In some cases the outline of the engulfed cell was retained but in others it showed

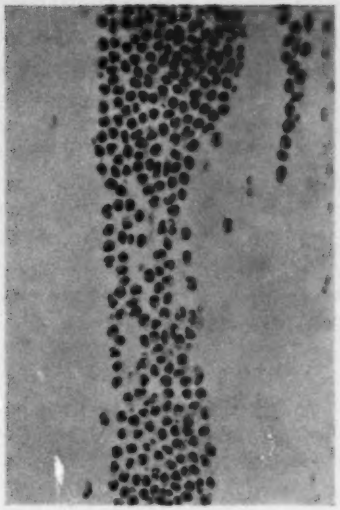

FIG. 1. Vaginal mucus smear, two days post-oestrum, showing leucocytes, monocytes and erythrocytes. ×220.

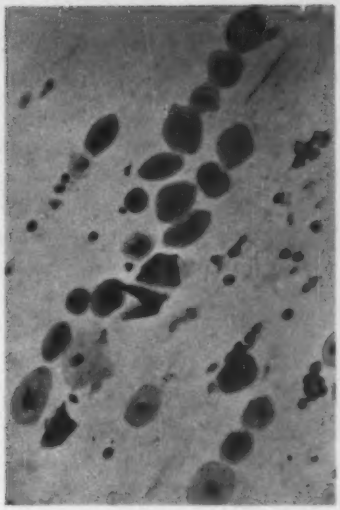

FIG. 2. Vaginal mucus smear, two days post-oestrum, showing keratinized cells, leucocytes and epithelial cells with engulfed elements. ×220.

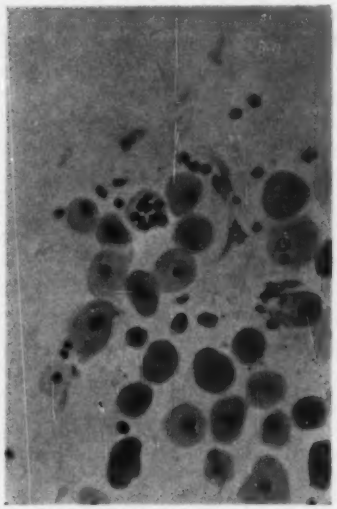

FIG. 3. Vaginal mucus smear, two days post-oestrum, showing epithelial cells with engulfed leucoytes and plasma cells. ×220.

FIG. 4. Vaginal mucus smear, three days post-oestrum, showing a sheet of epithelial cells, one of which has engulfed seven plasma cells. ×350.

signs of disintegration. The engulfed cells could be clearly seen in preparations viewed under the phase-contrast microscope.

Keratinized cells were never prominent. Those present were usually found in clusters, orange to red in colour, angular in outline, anuclear, or having a faintly staining nucleus (Fig. 2).

Occasionally tall columnar cells with a basal nucleus could be seen. These cells measured approximately 6 μ by 12 μ, the cytoplasm was faint blue and the nucleus stained intensely black; intracytoplasmic red granules were a feature. Erythrocytes appeared either at the time of post-oestrous bleeding or if minor trauma occurred during the collection of the sample. They stained bright orange. Mucus could be demonstrated in most of the smears; it stained blue.

Cellular Variation in Vaginal Mucus throughout the Oestrus Cycle

On the day of visible oestrus, cells were virtually absent from the vaginal mucus; those present were usually small epithelial elements, with occasional leucocytes and keratinized cells. The same lack of cells was occasionally apparent the day before oestrus and in some cases there would not be a high cell concentration before the third day after the onset of oestrus; it was usual to find smears lacking cells for approximately two to three days at this time. By the second or third day post-oestrum there was a moderate increase in the number of leucocytes, and this was immediately followed, about the third day, by the appearance of large numbers of epithelial cells. Epithelial cells predominated on the third or fourth day after the onset of oestrus and erythrocytes were usually evident at the same time. The epithelial cells at this time were often in sheets and leucocytes could be seen scattered between the cells. At this period in the oestrus cycle the number of epithelial cells was at its maximum and it was also now that the greatest number of engulfed elements could be demonstrated. During the remainder of the cycle epithelial cells were never completely absent and by careful searching it was usually possible to find single cells with included leucocytes throughout the 21 days of the cycle. Leucocytes increased in number from the third or fourth day post-oestrum, reaching their maximum concentration around the tenth day, but no regularity in the variation in the numbers present could be demonstrated.

Keratinized cells were seldom absent but there did not appear to be any specific stage in the cycle when they were present in maximum numbers. Mucus was present throughout; at oestrus, it appeared as a diffuse blue background turning into fine strands about the time post-oestrus bleeding ceased, on the third or fourth day.

DISCUSSION

In recent years there have been reports by Millar (1955), Kendrick, McKercher & Saito (1956), Kendrick, Gillespie and McEntee (1958), McClure (1957), McIntosh, Haig & Alexander (1954) and Greig, Bannister, Mitchell & Barker (1958) implicating viruses as associated factors in bovine reproductive

failure. Gamčík (1958) used the cellular picture of vaginal and preputial epithelial smears in an attempt to arrive at a better understanding of an infectious posthitis in bulls. He stated that there was, in infected animals, an increase of cell elements up to double the normal values, but he failed to define the normal value. It would assist a better understanding of infectious conditions in the bovine reproductive tract if the cytological factors of vaginal secretions are properly understood. Thus far there has been considerable variation in the reported examination of the cytology of vaginal mucus; this may have been due to the fact that samples of mucus were not taken from the same area of the tract and also because too few animals of normal history and equal age were examined. Cole (1930) and Brown (1944) stated that the histological changes occurring in the vaginal mucosa were most marked 1 to 2 cm. from the cervix and it seemed rational to base the present work on material aspirated from the anterior vagina.

Cole (1930), Hansel, Asdell & Roberts (1949), and Roark & Herman (1950) were the only workers to state specifically that they collected material from the anterior vagina when assessing the cellular pattern in cows. Cole reported that small epithelial cells were common in the anterior vagina and that large cells were found only in smears from the vestibular area. He did not define the cell sizes but the photographs accompanying his work suggest that his "large" cell is the same cell as was commonly found, during the present work, in smears from the anterior vagina of the heifers.

It was one of the characteristics of the present series of smears that large epithelial elements appeared as the predominant cell three to four days post-oestrum. There was agreement with Cole's finding that cornified cells were virtually absent at oestrus. Hansel and his colleagues (1949) found that epithelial cells predominated up to the third day post-oestrum and that cornified elements were absent at oestrus; this is in general agreement with the present findings.

Many epithelial cells, especially when this type was predominant post-oestrum, showed the presence of cells, engulfed or otherwise included; these were usually leucocytes, but other elements believed to be plasma cells were seen occasionally. This phenomenon was reported by Frei & Metzger (1926). Hansel *et al.* (1949) noted leucocytes adhering to the surface of epithelial cells in a smear prepared one day post-oestrum but did not suggest that they were engulfed. Papanicolaou (1945) illustrated the type of inclusions encountered during the present work. He showed the engulfment of leucocytes by epithelial cells of the human vagina, and considered that the phenomenon could be associated with non-malignant conditions including chronic cervicitis and *Trichomonas* infestation.

The inter- and intra-infiltration of epithelial cells by leucocytes could be regularly demonstrated in smears from healthy heifers and it is suggested that the phenomenon should be regarded as a normal physiological process. The elements believed to be plasma cells had typical eccentric nuclei but attempts to confirm the cell type using Pappenheim's selective stain were not successful because the suspect cells were seldom found.

SUMMARY

Epithelial cells and polymorphonuclear leucocytes were the predominant elements in the vaginal mucus of healthy virgin heifers.

At oestrus, the mucus was generally acellular; leucocytes appeared on the second or third day after oestrus. There were large numbers of epithelial cells by the third to fifth day but these quickly fell in number. Leucocytes were the characteristic cells from the fourth day onwards and reached maximum numbers by the tenth day after oestrus.

The engulfment of leucocytes by epithelial cells is regarded as a normal physiological phenomenon.

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GRISEOFULVIN FOR *TRICHOPHYTON MENTAGROPHYTES* INFECTION OF CHINCHILLAS

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Trichophyton mentagrophytes infection of chinchillas has been described in some detail by Blank, Byrne, Plummer & Avery (1953), Rowsell, Kennedy & Fischer (1954) and Hayes (1956). This infection of chinchillas is commonly known as "fur fungus", and the use of griseofulvin for treating it has also been described by McPherson (1960), La Touche (1960), Kelley and Anthony (1961) and Uvarov (1961).

Dermatophyte infections in a closed colony of any species of animal present many problems. Daily handling of each individual animal for topical treatment is time-consuming and, if many animals are involved, impossible. The use of topical treatment alone may even be of little value in dealing with an outbreak, in that clinically normal animals may harbour infection on the hair and so act as residual sources of reinfection. Spores of *T. mentagrophytes* may remain viable away from the host for many months (McPherson, 1957).

DESCRIPTION

In the three colonies described below it was decided, because of lack of success with a variety of topical treatments and because of the length of time that this treatment took, that oral griseofulvin therapy alone should be carried out. Whenever possible during the course of the outbreaks buildings and cages were disinfected by heat or chemical methods.

A diagnosis of *T. mentagrophytes* was made by cultural methods in a number of animals in each colony; the others were diagnosed only by visual examination.

The lesions varied in size and number. When infection was long-standing the lesions were extensive and the affected areas were denuded of hair; the skin was very inflamed and moist. Once infection was diagnosed on the premises, new cases were treated as soon as lesions were noted; generally the primary lesion occurred as a small, mildly inflamed area about 1 centimetre in diameter, covered by a thin growth of broken hairs. In most cases the primary lesions were found on the nose or near the eyes.

The three colonies were run commercially and it was not possible to keep controls, but in each ranch a number of animals had lesions of three to four months' duration, demonstrating the persistence of the disease.

Figs. 1, 2 and 3 show the length of infection before treatment and the length of treatment with griseofulvin. Griseofulvin was always given in a dose of 30 to 40 mg./kg. body weight daily by mouth. Tablets of griseofulvin of various strengths were prepared, to simplify administration. Most animals generally accepted the tablets spontaneously; the remainder would only accept them placed in or rubbed with a raisin. Treatment in colonies 1 and 2 was continued until a clinical cure was produced, but in colony 3 treatment was stopped after 28 days irrespective of clinical response. Nevertheless, in this colony clinical improvement continued as in colonies 1 and 2.

FIG. 1. Colony 1: length of infection before treatment and length of treatment with griseofulvin.

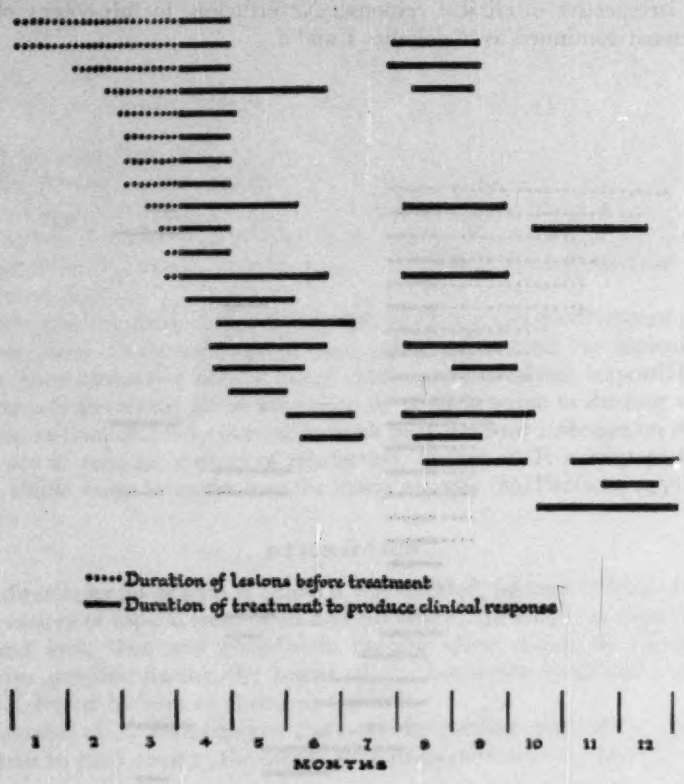

FIG. 2. Colony 2: length of infection before treatment and length of treatment with griseofulvin.

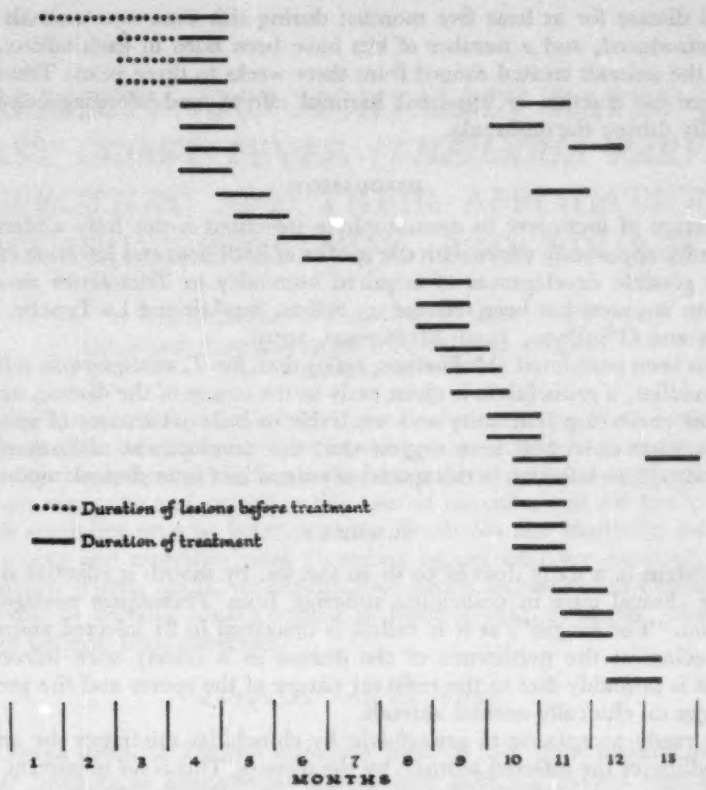

FIG. 3. Colony 3: length of infection before treatment and length of treatment with griseofulvin.

REINFECTION

Reinfection or relapse occurred in several animals, so that the clinical disease remained in the colonies some months after griseofulvin therapy was begun (see Table I). At the time of writing all three colonies have been free from

TABLE I

THE RELATIONSHIP BETWEEN THE NUMBER OF ANIMALS SHOWING RECURRENCE OF LESIONS AND THE DURATION OF INFECTION BEFORE TREATMENT WITH GRISEOFULVIN WAS BEGUN

	No. of animals	*No. in which re-infection occurred*
Animals in which lesions were present at least 3 months before griseofulvin treatment	6	3
Animals in which lesions were present 1-3 months before griseofulvin treatment	16	4
Animals in which lesions were present less than 1 month before griseofulvin treatment	59	20
TOTAL	81	27

clinical disease for at least five months; during this time new animals have been introduced, and a number of kits have been born in each colony. The age of the animals treated ranged from three weeks to three years. Treatment produced no reaction or apparent harmful effects, and breeding continued normally during the outbreaks.

DISCUSSION

The nature of immunity to dermatophyte infections is not fully understood. Immunity apparently varies with the species of both host and infecting fungus.

The possible development of acquired immunity to *Trichophyton verrucosum* infection in calves has been referred to (Sellers, Sinclair and La Touche, 1956; Lauder and O'Sullivan, 1958; McPherson, 1959).

It has been postulated (McPherson, 1960) that, for *T. mentagrophytes* infection of chinchillas, if griseofulvin is given early in the course of the disease, animals may fail to develop immunity and are liable to have recurrence of infection. The 81 cases described here suggest that the development of immunity to *T. mentagrophytes* infection in this species of animal is of little clinical importance.

SUMMARY

Griseofulvin in a daily dose of 30 to 40 mg./kg. by mouth is effective in producing clinical cure in chinchillas suffering from *Trichophyton mentagrophytes* infection. "Fur fungus", as it is called, is described in 81 infected animals in three colonies; the persistence of the disease in a colony once infection is present is probably due to the resistant nature of the spores and the presence of fungus on clinically normal animals.

The ready acceptance of griseofulvin by chinchillas minimises the amount of handling of the infected animals by the owners. This is an important point in relation to cross-infection from animal to man. Routine disinfection of the cages and premises would also seem to be important for eradication of the disease from a closed colony.

In the three colonies reinfection occurred irrespective of how long the lesions had been present before griseofulvin treatment was started.

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MORBIDITY AND MORTALITY RATES IN EAST COAST FEVER (*THEILERIA PARVA* INFECTION) AND THEIR APPLICATION TO DRUG SCREENING PROCEDURES

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The enzootic status of East Coast fever in much of eastern, central and southern Africa makes this infection one of the most important cattle diseases, economically, to the stock owner. The losses are both indirect and direct. The former arise from expensive and repetitive tick control measures and the latter from the high mortality rates in infected cattle. Textbooks cite morbidity rates of 100 per cent and mortality rates exceeding 95 per cent are reported from South Africa (Neitz, 1957), whereas in East Africa recoveries are frequent in Zebu cattle in enzootic areas (Barnett & Bailey, 1955). Protocols, however, have not hitherto been published.

MATERIAL AND METHODS

Parasite

The Muguga strain of *Theileria parva* was used. It has been maintained by alternate passages in *Rhipicephalus appendiculatus* ticks and Kenya high-grade cattle for at least ten years and is regarded as a typical strain.

Cattle

High-grade crosses of various breeds of European origin, reared in East Coast fever-free districts of Kenya, were exposed to the parasite at two to three years of age when they weighed approximately 200 kg. The cattle were housed in specially designed tick-free isolation stalls.

Exposure

Ten adult ticks (*R. appendiculatus*), collected as engorged nymphs from previously infected cattle, were placed on one ear of each animal, the ear being enveloped in a cloth bag (Bailey, 1960). The ticks were random samples from batches of proven high infectivity. Exposed cattle were examined regularly during the ensuing six weeks or until they died. Thin blood films and smears of biopsy samples of the parotid and prescapular lymph nodes were prepared daily, stained with Giemsa and examined. Infection was considered established when macroschizonts were observed in lymph node smears.

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RESULTS

Morbidity rate

Infections developed in 134 (87·6 per cent) of 153 cattle exposed.

Mortality rate

Deaths occurred in 128 (95·5 per cent) of the 134 cattle successfully infected.

DISCUSSION

The Muguga strain of *T. parva* has proved to be as virulent as the strains found in South Africa. Undoubtedly the type of cattle exposed influenced the mortality rate and exotic breeds are more susceptible than indigenous Zebus (Barnett & Bailey, 1955).

A cure for East Coast fever has yet to be discovered. Despite the extensive literature on drug screening, comparatively few well-designed therapeutic trials have been reported, but field nostrums against East Coast fever are legion. The case for the standardization of trials has been put forward by Barnett (1958).

The system of exposing high-grade cattle to a virulent strain of *T. parva*, as was used here, is a suitable method for the screening of potential therapeutic agents. Two effects can be measured, namely the suppression of the development of the parasite, following a treatment begun during the incubation period, and the recovery of infected animals following a treatment begun after infection is established. If four cattle are exposed and none becomes infected after a treatment initiated during the incubation period, the result is highly significant ($P < 0{\cdot}05$) (Table I).

TABLE I

MORBIDITY RATES SIGNIFICANTLY SMALLER THAN THE EXPECTED 87·6 PER CENT

Number of cattle exposed	*Probability*		
	2·5%	0·5%	0·05%
2	0	NS	NS
3	0	0	NS
4	1	0	0
5	2	1	0
6	2	2	1
7	3	2	2
8	4	3	2
9	5	4	3
10	5	5	4

NS = not significant

If as few as three proven infected cattle are treated and recover the result is likewise highly significant ($P < 0{\cdot}05$) (Table II).

The above technique, however, will only reveal "wonder" drugs. A treatment not absolutely effective might by chance be rejected as ineffective because a few of the treated cattle chanced to die. A treatment which lowered the mortality rate by half would still be welcomed by practitioners and, moreover, the detection of a slight degree of therapeutic activity in one compound might

TABLE II

CASE MORTALITY RATES SIGNIFICANTLY SMALLER THAN THE EXPECTED 95·5 PER CENT

Number of cattle infected	*Probability*		
	2·5%	0·5%	0·05%
2	0	0	NS
3	1	0	0
4	2	1	1
5	3	2	1
6	3	3	2
7	4	4	3
8	5	5	4
9	6	5	5
10	7	6	5

NS = not significant

lead to the exploration of further chemicals of that type and the discovery of one that is highly effective. Thus to limit to less than 1 in 20 the chance of rejecting as ineffective a treatment capable of halving the mortality, at least eight infected cattle would be required and seven would have to die.

Past experience has suggested that certain treatments which failed to cure did partially suppress infections when treatment was begun during the incubation period (Barnett, unpublished). The morbidity rate might therefore be a more sensitive indicator of therapeutic activity. A practical index would be its reduction from 87·6 per cent to 37·6 per cent. This would necessitate exposure of at least seven cattle and the development of infection in six before rejection to limit the chance to less than 1 in 20 of rejecting as ineffective a partially effective treatment.

SUMMARY

The morbidity and mortality rates of 153 Kenya high-grade cattle exposed to the Muguga strain of *Theileria parva* were 87·6 and 95·5 per cent respectively. Criteria for the assessment of chemotherapy trials are suggested.

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STUDIES ON DISINFECTION OF EGGS AND INCUBATORS. V: THE TOXICITY OF FORMALDEHYDE TO THE DEVELOPING EMBRYO

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The effect of exposing chick embryos of different ages to formaldehyde has been investigated on numerous occasions and variable results have been obtained. The majority of reports indicate that embryos cannot be fumigated with safety between the second and fifth days of incubation. Clarenburg and Romijn (1954), however, have shown that embryos can be fumigated on the second and third day, without affecting hatchability, by using approximately 5 fluid ounces of formalin per 100 cubic feet; conversely reports have been received from commercial hatcheries of damage having occurred in embryos exposed at a stage of development as advanced as ten days when comparable amounts of formalin were used.

These varied reports on the likelihood of embryonic damage resulting from the use of formaldehyde are explained when the factors which influence the extent of the damage sustained are taken into account. These have been stated by Wilson (1951) and Clarenburg and Romijn (1954) to be the vitality of the embryo and the concentration of formaldehyde to which they are exposed during the fumigation period. The latter is not solely dependent on the amount of formaldehyde initially liberated, as a varied degree of loss can occur as a result of ventilation effects (Harry, 1954*a*). Clarenburg and Romijn, for example, stated that ventilation losses were marked in the type of incubator used for their tests.

The present investigation has been made using the highest concentration of formaldehyde normally generated, namely from 4½ fluid ounces formalin per cubic feet, fumigation being done in an incubator in which loss of formaldehyde was restricted to a minimum. The influence of the degree of vitality of the embryos on their susceptibility to damage by formaldehyde was investigated by fumigating eggs which had been stored for various periods before setting and which had originated from flocks of different breed, age and genetical history.

In addition to the effect of formaldehyde on embryonic mortality the effect on production of malpositions, malformations and delay in hatching was also investigated.

METHODS

Initial tests were made on groups of 80–100 eggs which were incubated in a fully loaded 6,000 egg Stevens incubator of 130 cubic feet capacity. The eggs were transferred for fumigation at the appropriate age to an identical incubator which was kept at the same temperature. After the eggs were transferred the fans of the fumigating incubator were stopped and the outlet ventilators closed. Because of the relatively small number of eggs fumigated on each occasion no evidence was obtained that the embryos were deprived of oxygen during the fumigation period as a result of the restriction of ventilation. Formaldehyde was generated from 4½ fluid ounces formalin per 100 cubic feet; permanganate was added in the ratio of 2: 3 parts of formalin. After 20 minutes a strong solution of ammonia was added to the permanganate residues, in an amount equal to half that of formalin used. The fans were then restarted and the outlet ports were opened. The purpose of ammonia neutralization was to facilitate removal of the fumigated eggs (Harry, 1954*b*). After a further ten minutes the eggs were removed and were returned to the original incubator for the completion of incubation.

The first group of eggs was transferred on the first day of incubation, the second group on the second day, this process being repeated until embryos of ages up to 18 days had been exposed. The following treatments were applied to control groups: (1) incubated without transfer or fumigation; (2) transferred on the third day for a 30-minute period without fumigation; and (3) fumigated on the fifth day without final ammonia neutralization.

At the end of the incubation period all unhatched embryos were examined to determine whether they were late-hatching or, if dead, the time of death, and any abnormalities were recorded. The eggs used in these tests were obtained from a flock of White Leghorn × Barred Rock. The Barred Rock line had been closely inbred (brother × sister mating) for 11 generations.

As a result of these tests further series of tests were made using larger numbers of eggs per group to investigate in greater detail the effects of fumigating eggs between the first and ninth day of incubation. These tests were made as described previously, except that a control group of eggs was transferred to the fumigating incubator for 30 minutes and returned for normal incubation before the eggs of the test group were fumigated. In this manner each group of eggs fumigated could be compared with a group which, apart from exposure to formaldehyde, had been exposed to the same conditions throughout. The eggs for these tests were obtained from four different flocks, as indicated in Table III.

Further tests were made in the same manner on embryos from flocks with either a poor or good hatchability performance which were fumigated on the third or ninth day of incubation. Tests were also made on embryos from a single flock, the eggs of which had been stored at temperatures fluctuating between 65° and 70°F for periods ranging from 1–11 days before setting. These embryos were fumigated on the third day of incubation.

RESULTS

The tests on the embryos from the White Leghorn × Barred Rock flock showed,

as indicated in Table I, that hatchability was unaffected when the embryos were fumigated before the third and after the eighth day of incubation. Embryonic mortality was particularly high when fumigation was carried out at the beginning and end of this period. Some effect was noted, however, on embryos fumigated between the eighth and 14th days, in that the numbers of malpositioned embryos increased. Fumigation on the second day, although it did not affect the overall hatchability, produced a high proportion of malformed embryos. The eight malformed embryos recorded in Table I all had beak deformities with either the lower beak longer than the upper one, or with the beak crossed; four were without eyes; three had only one eye and two were without a cranium. Three embryos with similar malformations were produced in the group fumigated on the third day of incubation. (Fig. 1).

TABLE I

THE EFFECT OF EXPOSING EMBRYOS AT DIFFERENT STAGES OF DEVELOPMENT TO FORMALDEHYDE. EGGS ORIGINATING FROM A WHITE LEGHORN × BARRED ROCK FLOCK

Days of incubation fumigated	*Age of embryo (days)*	*No. of fertile eggs per group*	*No. malpositioned*	*No. malformed*	*No. hatching late*	*% hatched fertile*
1	<1	76	4	0	8	68·4
2	1	73	5	8	1	67·1
3	2	77	2	3	3	16·9
4	3	81	4	0	2	30·9
4	3	89	11	1	4	46·1
5	4	87	4	0	6	46·0
5	4	83	4	0	8	41·0
*5	4	80	2	0	0	23·8
5	4	69	3	0	2	20·8
*5	4	67	3	0	0	39·0
6	5	74	1	0	0	27·0
8	7	83	11	1	7	14·5
10	9	91	14	0	8	64·9
13	12	87	12	0	4	70·1
15	14	79	9	0	0	65·8
17	16	78	2	0	0	77·0
19	18	81	7	0	2	70·4
† –	–	80	5	1	9	65·0
‡ –	–	85	6	0	5	71·8

* Formaldehyde not neutralized with ammonia.
† Transferred to fumigating incubator on the third day but not fumigated.
‡ Incubated without transfer or fumigation.

Determination of the time of death of the embryos in the tests showed, as indicated in Table II, that the peak of mortality in embryos fumigated between the third and ninth day coincided with the fumigation period and in some cases extended over the subsequent two to three days. This effect was also noted in the subsequent tests as detailed in Tables IV, VI and VIII.

The control tests indicated that the neutralization of formaldehyde with ammonia had no consistent effect on hatchability. The hatchability of the group of eggs which was transferred to the test incubator on the third day without fumigation and the groups which were fumigated after the eighth and before the third day were within the range of hatchability of all eggs from this particular flock which had been incubated normally during the period over which the tests were made.

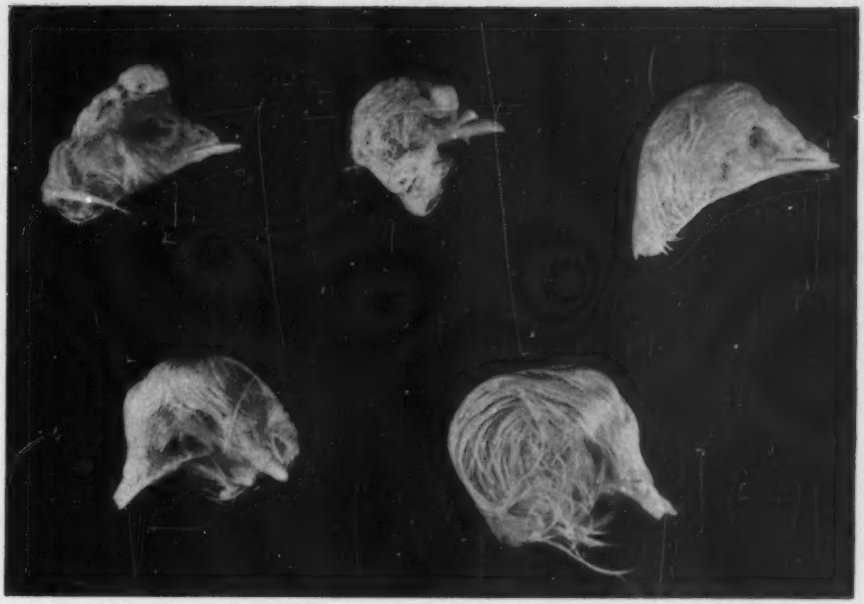

FIG. 1. Heads of embryos which had died at the termination of the incubation period, showing the various types of malformation produced by exposure to formaldehyde during the second or third days of incubation. L to R top: (1) and (2) no cranium, short upper beak; (3) short upper beak, no eyes; bottom: (4) two heads partially fused; (5) crossed beak, no eyes.

TABLE II

MORTALITY AND AGE DISTRIBUTION OF GROUPS OF EGGS REFERRED TO IN TABLE I

Day of incubation fumigated	*Mortality and age distribution % fertile eggs (Days)* 1–3	4–5	6–7	8–9	10–12	13–15	16–18	19–21
1	7·9	0	1·3	0	0	2·6	2·6	6·5
2	10·9	0	1·4	0	0	0	7·0	12·6
3	63·6	0	2·6	1·3	0	1·3	2·6	7·8
4	58·0	0	0	1·2	0	0	0	7·4
4	25·8	0	0	0	1·1	3·4	6·7	12·4
5	10·3	13·8	1·1	0	1·1	1·1	2·2	9·2
5	10·8	24·1	1·2	0	0	0	1·2	12·0
*5	12·5	48·8	3·8	0	0	0	2·5	8·8
5	13·0	55·1	2·9	0	0	0	0	5·8
*5	11·9	38·8	3·0	0	0	1·5	3·0	3·0
6	10·8	58·1	1·4	0	0	0	6·8	2·8
8	7·2	0	43·4	1·2	1·2	2·4	0	15·7
10	5·5	1·1	1·1	2·2	1·1	0	2·2	15·4
13	6·9	0	1·1	0	0	0	3·4	13·0
15	8·9	0	0	0	0	0	5·1	19·0
17	11·5	0	1·3	0	0	0	0	5·1
19	11·1	1·2	1·2	0	0	0	0	10·8
† –	10·0	1·3	1·3	0	1·3	0	0	10·4
‡ –	8·2	1·2	1·2	0	1·2	0	1·2	10·8

* Formaldehyde not neutralized with ammonia.
† Transferred to fumigating incubator on the third day but not fumigated.
‡ Incubated without transfer or fumigation.

TABLE III

THE EFFECT OF EXPOSING EMBRYOS AT DIFFERENT STAGES OF DEVELOPMENT TO FORMALDEHYDE AS SHOWN BY COMPARISON WITH SIMILARLY TREATED UNFUMIGATED EGGS

Day of incubation fumigated	*No. of fertile eggs per group*	*Flock of origin*	*No. mal-positioned*	*No. mal-formed*	*No. hatching late*	*% hatched fertile*
*–	87	R × WL	5	0	1	64·4
3	86	"	7	0	2	42·0
–	150	WL × R	9	0	3	68·7
3	173	"	5	2	6	72·9
–	90	WL (1)	16	0	3	50·0
4	95	"	2	0	3	55·8
–	68	WL × R	2	0	0	58·8
4	57	"	2	1	2	31·5
–	59	WL × R	5	1	3	52·5
†4	63	"	4	6	6	42·8
–	142	WL (1)	8	0	6	64·1
5	139	"	10	0	1	59·7
–	92	WL × R	10	1	1	60·9
5	86	"	9	0	6	61·6
–	88	R × WL	0	0	0	73·9
6	91	"	5	0	1	78·0
–	85	WL (2)	15	0	5	55·3
8	85	"	8	0	2	51·8
–	68	WL × R	6	0	2	55·9
9	71	"	8	0	1	57·8

* Indicates unfumigated controls.
† Formaldehyde not neutralized by ammonia.
R = Rhode Island Red.
WL = White Leghorn.

TABLE IV

MORTALITY AND AGE DISTRIBUTION OF GROUPS OF EGGS REFERRED TO IN TABLE III

Day of incubation fumigated	*Mortality and age distribution % fertile eggs (Days)*							
	1–3	4–5	6–7	8–9	10–12	13–15	16–18	19–21
*–	15·4	0	1·1	0	1·1	1·1	1·1	13·2
3	27·6	1·2	2·4	0	0	6·0	3·6	15·6
–	10·7	0	2·0	0	2·0	2·7	0·7	11·4
3	27·8	0	1·2	0	0·6	0·6	0·6	5·8
–	6·6	3·3	0	0	1·1	2·2	3·3	29·7
4	13·7	0	0	0	4·2	2·1	3·2	17·9
–	31·5	4·5	1·5	0	0	0	1·5	3·0
4	46·8	9·0	1·8	1·8	0	3·6	0	3·6
–	22·1	1·7	0	1·7	1·7	0	0	15·9
‡4	28·8	4·8	0	0	0	1·6	0	12·8
–	4·9	5·6	0·7	0	0	2·1	3·5	14·7
5	7·9	8·6	0·7	0	0·7	0·7	2·8	18·0
–	5·5	2·2	1·1	2·2	1·1	4·4	2·2	19·8
5	7·2	1·2	0	1·2	0	0	0	22·8
–	8·0	0	0	0	0	0	0	18·2
6	9·9	1·1	0	0	0	1·1	0	8·8
–	6·0	3·6	1·2	0	0	2·4	4·8	21·6
8	10·6	4·8	0	0	0	2·4	0	28·2
–	19·5	9·0	0	0	0	1·5	3·0	9·0
9	14·0	1·4	0	0	0	1·4	0	23·8

* Indicates unfumigated controls.
‡ Formaldehyde not neutralized by ammonia.

The tests which were repeated on embryos incubated from three to nine days, as shown in Table III, again indicated that a high degree of embryonic mortality was produced by exposure to formaldehyde on the third and fourth days of incubation. With eggs from the particular flocks used in these tests, however, no effect was noted in embryos fumigated on and after the sixth day. Comparison of the results obtained with embryos originating from different flocks used in this series and the previous series of tests showed that embryos originating from different flocks varied in their susceptibility to the effects of formaldehyde. It was found that the degree of susceptibility of the embryos of any particular flock was not invariably related to its hatchability performance as shown by the hatchability of the control groups.

The effect of the flock of origin of the embryos on their susceptibility to damage by formaldehyde was demonstrated more clearly in the tests detailed in Table V. The mortality age distribution of the embryos failing to hatch from the White Leghorn × Rhode Island Red flock which were fumigated on the ninth day, as shown in Table VI, indicated that although hatchability, as compared with the control group, was not adversely affected, the peak of embryonic mortality of the fumigated eggs occurred at and during the two days subsequent to fumigation.

The final series of tests made on embryos originating from a single flock, involving newly laid and stored eggs, showed (Table VII) that embryonic mortality resulting from exposure to formaldehyde on the third day of incubation was significantly higher in the eggs which had been stored. The poor

TABLE V

THE EFFECT OF EXPOSING EMBRYOS ORIGINATING FROM DIFFERENT FLOCKS TO FORMALDEHYDE

Day of incubation fumigated	*No. of fertile eggs per group*	*Flock of origin*	*No. mal-positioned*	*No. mal-formed*	*No. hatching late*	*% hatched fertile*
*–	12	R	0	0	2	58·3
–	34	BL	3	2	2	67·6
–	36	BR	10	3	2	33·3
–	38	WL × R	2	1	0	73·6
9	15	R	0	0	1	0
9	33	BL	2	0	0	0
9	35	BR	3	0	0	14·2
9	36	WL × R	0	0	0	83·3
–	47	R	7	0	5	59·5
–	10	BL	1	0	0	90·0
–	21	BR	5	3	2	38·0
–	92	WL	3	1	3	52·1
3	43	R	2	1	0	0
3	13	BL	0	0	0	0
3	22	BR	1	1	1	9·1
3	97	WL	1	0	0	2·2

* Indicates unfumigated controls.
R = Rhode Island Red.
BL = Brown Leghorn.
BR = Barred Rock.
WL = White Leghorn.

TABLE VI

MORTALITY AND AGE DISTRIBUTION OF GROUPS OF EGGS REFERRED TO IN TABLE V

Day of incubation fumigated	*Flock of origin*	*Mortality and age distribution % fertile eggs (Days)* 1–3	4–5	6–7	8–9	10–12	13–15	16–18	19–21
*–	R	8·3	0	0	0	8·3	0	0	8·3
–	BL	2·9	0	0	0	0	8·7	0	14·5
–	BR	5·6	0	0	2·8	0	2·8	11·2	39·2
–	WL × R	18·4	0	0	0	0	2·6	2·6	2·6
9	R	6·7	6·7	20·1	46·9	6·7	0	0	6·7
9	BL	0	6·1	18·2	48·5	15·2	6·1	0	6·1
9	BR	5·7	11·4	22·8	25·7	8·6	0	0	14·3
9	WL × R	5·6	0	0	8·3	72·2	0	0	5·6
–	R	12·6	0	2·1	0	0	0	0	14·7
–	BL	0	0	0	0	0	0	0	10
–	BR	4·8	0	0	0	0	9·6	9·6	28·8
–	WL	28·3	1·1	2·2	0	0	0	1·1	11·0
3	R	92·0	2·3	2·3	0	0	0	4·6	0
3	BL	92·4	7·7	0	0	0	0	0	0
3	BR	76·5	0	0	4·5	0	0	0	4·5
3	WL	90·1	2·2	2·2	1·1	0	0	1·1	1·1

* Indicates unfumigated controls.
R = Rhode Island Red.
BL = Brown Leghorn.
BR = Barred Rock.
WL = White Leghorn.

hatchability shown by the control groups was normal for this particular flock.

The incidence of clubbed down amongst the dead embryos in all the tests made was the same as that in the control groups, i.e. less than one or two per group, with the exception of one test in which embryos were fumigated on the

fourth day. The isolated occurrence of this, however, suggests that it was unrelated to the fumigation procedure.

TABLE VII

THE EFFECT OF EXPOSING EMBRYOS TO FORMALDEHYDE ON THE THIRD DAY OF INCUBATION. THE EMBRYOS ORIGINATED FROM A SINGLE FLOCK OF WHITE LEGHORN, BUT FROM EGGS EITHER NEWLY LAID OR STORED

Group	*Duration of storage period (days)*	*No. of fertile eggs*	*No. malpositioned*	*No. malformed*	*No. hatching late*	*% hatched fertile*
Control	11	24	3	3	0	45·8
	10	29	3	1	0	44·8
	2	25	3	2	0	48·0
	1	26	0	0	2	46·1
Fumigated	11	21	1	0	0	14·2
	10	25	1	1	0	16·0
	2	22	0	0	1	36·3
	1	27	1	0	0	37·0

TABLE VIII

MORTALITY AND AGE DISTRIBUTION OF GROUPS OF EGGS REFERRED TO IN TABLE VII

		Mortality and age distribution % fertile eggs (Days)							
Group	*Storage period (days)*	1–3	3–5	6–7	8–9	10–12	13–15	16–18	19–21
Control	11	21·0	0	4·2	0	4·2	4·2	0	21·0
	10	30·6	3·4	3·4	0	0	3·4	0	13·6
	2	16·0	8·0	4·0	0	4·0	0	8·0	12·0
	1	22·8	0	0	7·6	3·8	0	0	11·4
Fumigated	11	72·0	0	0	4·8	0	0	0	9·6
	10	56·0	12·0	0	0	0	4·0	0	12·0
	2	45·0	9·0	0	0	0	0	0	4·5
	1	33·3	3·7	0	0	0	0	3·7	22·2

DISCUSSION

The tests have shown that if the maximum recommended levels of formalin and permanganate are used in an incubator in which leakage of formaldehyde is reduced to a minimum, an increase in embryonic mortality can occur in embryos exposed to formaldehyde on the third to ninth days of incubation. Such mortality has been shown to be influenced by the flock of origin of the eggs and the period for which they have been stored before incubation. There was an indication that the incidence of malpositioned embryos increased when embryos were exposed to formaldehyde at periods beyond the ninth day and up to the 14th day. The production of embryonic mortality, malpositioning or malformation by fumigation, however, did not invariably reduce hatchability below that of unfumigated controls and it is considered that this indicated that in many cases the embryos affected were those of subnormal vitality which would have failed to hatch under normal incubation conditions. Similarly, a relatively low hatchability with a high early embryonic mortality was usually

associated with a decrease in the number of late-hatching chicks. Differences in the susceptibility of embryos of a particular flock to the toxic effect of formaldehyde were not found to be invariably associated with hatchability performance, however, and if susceptibility can be considered to be an index of the vitality of the embryos, the poor hatchability of certain of the pure breeds referred to in Tables V and VII must be attributed to other causes.

These results indicate the advantage to be gained from the use of the method of preincubation fumigation as described by Lancaster, Gordon and Harry (1954), as even when eggs are set at weekly intervals, fumigation in the incubator can in some instances adversely affect hatchability. Furthermore, if the amounts of formaldehyde used in these tests are generated in an incubator operated at a normal ventilation rate, concentrations of formaldehyde intolerable to hatchery personnel escape into the hatchery room. In a correctly designed fumigation chamber this drawback to the use of formaldehyde can be avoided.

SUMMARY

(1) Tests have shown that hatchability can be adversely affected when embryos are exposed for 20 minutes on the third to ninth days of incubation to formaldehyde when this is generated from formalin and permanganate at a rate of 4½ fluid ounces of formalin per 100 cubic feet in an incubator from which escape of formaldehyde is reduced to a minimum.

(2) The degree to which hatchability is depressed by exposure to formaldehyde has been shown to vary considerably with the particular flock from which the embryos originate and with the duration of the preincubational egg storage period.

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SEROLOGICAL IDENTIFICATION OF OX, BUFFALO, GOAT AND DEER FLESH

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Although the identification of the flesh of closely related animals is not of practical importance in most countries, the game-protection laws of Ceylon, the local by-laws and the criminal cases of theft and slaughter of cattle have necessitated the adoption of some method which would identify with certainty the blood and flesh of the related species, ox, buffalo, goat and deer. The evidence given by veterinary surgeons, based on physical characteristics, is accepted in our Courts of Law but is open to challenge by the defence.

The direct precipitin test fails to distinguish between closely related species, as was shown by Nuttal (1904), Proom (1943), and Duncan Taylor (1952). Attempts to obtain a specific antiserum by absorption of the heterologous antibodies were not regularly successful, according to Landsteiner (1947). However the absorption technique worked out in this laboratory has overcome the difficulties encountered by previous workers, and this paper describes a simple technique used to distinguish between ox, buffalo, goat and deer flesh.

Members of the Bovidae and Cervidae had earlier been differentiated with respect to their blood sera by Baier and Wolfe (1942), who employed a quantitative precipitin technique. As they pointed out, however, the disadvantage in their test was that its operation was time-consuming. Weitz (1952) and Weitz and Glasgow (1956) employed a combination of the precipitin and inhibition tests to identify closely related animals. Their method of preparation of the antisera was unique in that they obtained non-specific antisera of high titres by repeated intramuscular injections of alum-precipitated serum, and then absorbed out the heterologous antibodies with no resultant loss of titre. The precipitin test served to distinguish between ox and sheep or goat, but to differentiate ox from buffalo these authors used the inhibition test.

PREPARATION OF ANTISERA

Antigen—alcohol-precipitated protein

Twenty ml. of blood serum was diluted with 80 ml. normal saline and 150 ml. absolute alcohol was added gradually. The mixture was well shaken and kept at room temperature for a few hours. It was then kept overnight in the refrigerator and filtered at the pump the following day. After some time the protein layer was found to crack up; it could be lifted off the filter paper, transferred to a Petri dish, broken up with a spatula and dried in a

desiccator. The dry protein could be kept for a number of years without deterioration if stored in a desiccator. One hundred ml. serum yielded approximately 7 g. protein.

Injection material

Protein (0·7 g.) was ground to a very fine powder in a mortar and suspended in 70 ml. saline. This material was sufficient for two rabbits, duplication being necessary since sometimes a rabbit did not produce high-titred antiserum and since an occasional rabbit produced an antiserum which could not be rendered specific by absorption.

Method of immunization

1st day: 5 ml. } protein suspension injected
2nd day: 10 ml. } intraperitoneally into
3rd day: 15 ml. } each rabbit weighing 2 kg.

By about the tenth day experimental bleedings were started and the antiserum tested against the corresponding normal serum at dilutions of 1 in 200, 1 in 1,000, 1 in 5,000 and 1 in 8,000.

Generally the maximum titre produced by this method was 1 in 8,000 to 1 in 10,000; the 1/200 dilution gave a strong reaction within a minute. This occurred about 10 to 12 days after the last injection. The rabbits were then starved for 24 hours and bled to death, the blood being collected in sterile test-tubes. After about an hour the clotted blood was ringed with a sterile glass rod. The tubes were left at room temperature for a few hours and then overnight in the refrigerator. The following day the antiserum was pipetted out, centrifuged and placed in ampoules under aseptic conditions. The ampoules were stored in the refrigerator.

Any rabbit that produced a low-potency antiserum was discarded and not used again for injections of the same or any other protein.

Contrary to accepted criteria, the potency of the antiserum was judged by the intensity of the reaction with the 1/200 dilution and not merely by positive reactions with the higher dilutions. During the test bleedings on individual rabbits and in the testing of the numerous antisera prepared here, a fairly wide quantitative variation in the reactions with the 1/200 dilution was observed, whereas the higher dilutions all gave similar reactions. This was observed when the antiserum was tested not only against the homologous antigens but also against the heterologous related antigens.

Absorption technique

Dilutions of 1/200 of normal ox, buffalo, goat and deer sera were used for absorption. Preliminary titrations were carried out to determine the amount of serum required to absorb out completely the corresponding heterologous antibody. For example, for one anti-ox serum prepared in this laboratory, absolute specificity against buffalo was obtained by the addition of 1 in 200 dilution of normal buffalo serum in the proportion three parts of anti-ox serum to one of buffalo serum dilution. This absorbed anti-ox serum still reacted with the 1 in 200 dilutions of goat and deer sera. When goat serum

(1/200) was added to anti-ox serum in the ratio 1:3, however, an anti-ox serum resulted which was specific against goat and deer, but not against buffalo.

This particular anti-ox serum was rendered specific against buffalo, goat and deer when 1 in 200 dilutions of the corresponding sera were added in the proportion 3:1. However, to obtain an anti-ox serum specific against all three, it was not found necessary to add the combined three volumes of the diluted sera to three volumes of antiserum. Similar results were obtained with anti-buffalo, goat and deer sera.

Table I lists the titrations of the unabsorbed antisera against 1 in 200 dilutions of normal sera. Table II gives the results obtained when the absorbed

TABLE I

TITRATIONS OF UNABSORBED ANTISERA AGAINST HOMOLOGOUS AND HETEROLOGOUS SERA

	1 *in* 200 *Dilution of normal serum*			
Antiserum	*Ox*	*Buffalo*	*Goat*	*Deer*
Ox	++++++	++++	+++	++
Buffalo	++++	++++++	+++	+++
Goat	+++	+++	+++++	++++
Deer	++	++	++++	+++++

The degree of precipitation based on visual examination varied from a maximum indicated by ++++++ to a minimum indicated by ++.

TABLE II

TITRATIONS OF ABSORBED ANTISERA AGAINST HOMOLOGOUS AND HETEROLOGOUS SERA AND FLESH EXTRACTS

	1 *in* 200 *Dilution of normal serum flesh in normal saline*			
Absorbed antiserum	*Ox*	*Buffalo*	*Goat*	*Deer*
Ox with B — + 200, G — + 200, D — 200; 12 : 4 : 2 : 1	+	—	—	—
Buffalo with Ox — + 200, G — + 200, D — 200; 12 : 4 : 2 : 1	—	+	—	—
Goat with Ox — + 200, B — + 200, D — 200; 12 : 4 : 2 : 1	—	—	+	—
Deer with Ox — + 200, B — + 200, G — 200; 12 : 4 : 2 : 1	—	—	—	+

The proportions of the heterologous sera were kept constant at 4 : 2 : 1 for uniformity. They could be varied within limits without affecting the ultimate result, which is the specificity.

\+ indicates positive reaction.

— indicates negative reaction.

antisera were tested against the normal sera dilutions and against the saline extracts of the flesh of the four animals.

For the preparation of the absorbed antiserum, the antiserum and the heterologous serum dilutions were introduced into a small tube using a graduated pipette. The mixture was well shaken for a few minutes, allowed to stand at the room temperature (28°C) for 15 minutes and then centrifuged at 4,000 rev./min., when a clear supernatant was obtained. The precipitin test was carried out in capillary tubes and the results noted after 20 minutes at room temperature.

The absorbed specific antiserum was lower in potency than the unabsorbed with respect to the 1 in 200 homologous serum dilution, but the decrease was not so appreciable as to render the antiserum valueless for practical purposes. A negative reaction was obtained not only with the 1 in 200 dilution but with all dilutions of the normal heterologous sera, i.e. the absorbed antiserum was absolutely specific.

Preparation of flesh extracts

Flesh samples for identification should have no preservative added to them. A few small pieces of the flesh, preferably free from fat, were placed in a small tube and normal saline added. The mixture was gently macerated with a glass rod until the extract was coloured pink or red. This was left overnight in the refrigerator and centrifuged the following day. The clear liquid could be tested out directly against the absorbed antisera but, as a rule, it was filtered prior to testing as it sometimes became turbid on standing in the capillary tubes during the test. A simple but effective means of filtration is to allow the extract to seep very slowly through a plug of filter paper inside a pipette.

The precipitin test

The filtered extract was drawn up halfway into a capillary tube about 3½ inches long. A drop of antiserum placed on a porcelain tile was then drawn up the same capillary and the top of the tube was sealed over a Bunsen flame. Similar tubes were prepared against the other antisera. The tubes were kept in the vertical position for 20 minutes and examined by transmitted light against a black background, when a white precipitate was observed at the interface of the two liquid layers. It was necessary to dilute the stronger extracts before testing, as otherwise the precipitate settled down quickly and simulated a turbid lower layer.

No identification was possible when the flesh had been subjected to heat treatment in the form of cooking, excessive "smoking" or sun-drying, or to the action of formalin. The majority of the samples submitted to this laboratory for examination were received in a partially putrefied condition; nevertheless, successful identification of the species was made in every case. Filtration after centrifugation was found to be necessary in order to obtain clear extracts and to eliminate turbidity after the capillary tubes were set up.

Of 71 samples of flesh analysed, 17 were ox, 13 buffalo, 2 goat and 39 deer.

SUMMARY

The identification of flesh presents special problems in Ceylon. Closely related species cannot be differentiated by the direct precipitin test. The paper describes a simple absorption technique which is used in this laboratory for the identification of flesh of the closely related species: ox, buffalo, goat and deer.

This absorption method differs from those tried out by other workers in the method of preparation of the antisera, the dilution of the heterologous sera used for absorption and the time allowed for complete absorption. The success of this method, we feel, is due mainly to the method of immunization which produces antisera of high titres which are absolutely specific with respect to unrelated species.

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THE ARTERIAL CIRCULATION OF THE KIDNEY OF A YOUNG ELEPHANT (*ELEPHAS INDICUS*)

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Department of Veterinary Anatomy, Royal (Dick) School of Veterinary Studies, University of Edinburgh

The vinylite and acid corrosion method is a most useful technique for the study of the arterial system of the kidney and has already been described (Grahame, 1944). There have, however, been so many enquiries concerning the application of this method that some details of its practical application are given here in the hope that they may prove helpful in making reasonably good vascular casts.

The organ must be fresh, healthy and uninjured. Injury is usually caused when the kidney is removed from the body. Most organs in small animals are better injected *in situ*. It is useful and expedient to keep several coloured and uncoloured solutions of vinylite in stock. Uncoloured and coloured 18 per cent acetone solutions kept in glass-stoppered winchester bottles are suitable strengths for dilution. The colours should be slightly darker than those required for the cast. (The colouring agents are dyes manufactured by Imperial Chemical Industries Ltd.). The finer arteries receive 12 per cent solution, veins 8·5 per cent solution, tubes (i.e. ureter, calyx, etc.) 18 per cent solution.

The range of pressure is variable, 80–600 mm. of mercury for arteries, 100–200 mm. for veins, and 80–100 mm. for tubes (ureter), and some experience is needed to assess the appropriate pressures for the different organs.

The longer the organ is kept in acid (HCl) the more fragile the smaller structures become. Frequent use of a fine water-jet will remove much disintegrating tissue and therefore lessen the time in acid.

The cast should not be allowed to dry. It may be kept for very long periods in 5 per cent formol solution. Casts have been kept in our museum for over 30 years without showing any deterioration.

THE RIGHT KIDNEY OF THE INDIAN ELEPHANT

A description of the arterial system, calyces and ureter of the left kidney of a 36-year-old Indian elephant was given by Grahame (1944). The right kidney of an eight-year-old female elephant which died in the Edinburgh Zoological Gardens has provided an opportunity to investigate further, by means of a

cast made by the vinylite and acid corrosion method, the general concept of these structures. The opposite kidney would show some slight deviations but these should be of no material consequence to the general plan of circulation.

In the various works on this subject, there is still some confusion concerning the nomenclature. Most of the confusion arises because the terminology used for the primitive kidney is not adopted for the more specialized kidney. The kidneys of sea mammals are multilobular or multipyramidal, each lobule being considered an independent unit. The specialized kidneys of the horse, dog, sheep, etc., are of the unipyramidal type. In these animals the renal pyramids have linked up, giving the impression of a single pyramid. The kidneys of the human, ox, pig, bear, etc., are between the above types; two or more renal pyramids or renculi (Guzsal, 1960) link up and form a multipyramidal type of kidney, the numbers of lobules being considerably reduced. The nomenclature used for the arterial circulation of a multilobular kidney ought to be used for the kidney of any mammal, whether unipyramidal or multipyramidal, and it is applied here to that of the elephant.

The renal arteries

The renal artery of the elephant (Fig. 1), like that of most mammals, divides into a dorsal and ventral artery, each supplying their respective halves of the kidney without anastomosing. The pattern of each of these branches is similar. The branching of the ventral and dorsal vessels follows in sequence—interlobar, lobar, interlobular, arciform, lobular, afferent, glomerular and efferent. There are variations in the number of branches. The ventral vessel has more branches than the dorsal vessel and it gives off a ureteric artery.

This pattern of renal vascularization is also common to most mammals. The complete independence of the dorsal and ventral vessels supplying the respective two halves of the kidney suggests a separate origin from the same anlage. Each half so differentiated consists of several recognizable lobules, the number varying by not more than one or two lobules. Even when lobules are not visibly discernible, a cast of the vessels does provides a distinct picture of the vascular lobulation and of the complete absence of any anastomosis between the vessels of the lobules or the vessels within the lobule. A lobule is usually supplied by two vessels, one vessel to one surface of the lobule, the other to the opposite surface. When there is only one lobar vessel, it divides into dorsal and ventral vessels which follow the plan given above. There is sometimes a slight variation in the vascular supply, especially to a small lobe, when the interlobar vessel instead of dividing into dorsal and ventral vessels provides circumferential branches, which, however, still have a similar pattern of distribution.

The ureter

The ureter (Fig. 1) and its divisions are almost a replica of those of the older animal described previously. There is a swelling just before it divides into three major calyces, anterior, middle and posterior; from these are derived ten minor calyces, each terminating by forming a middle and two terminal recesses, which is similar to the formation in the horse.

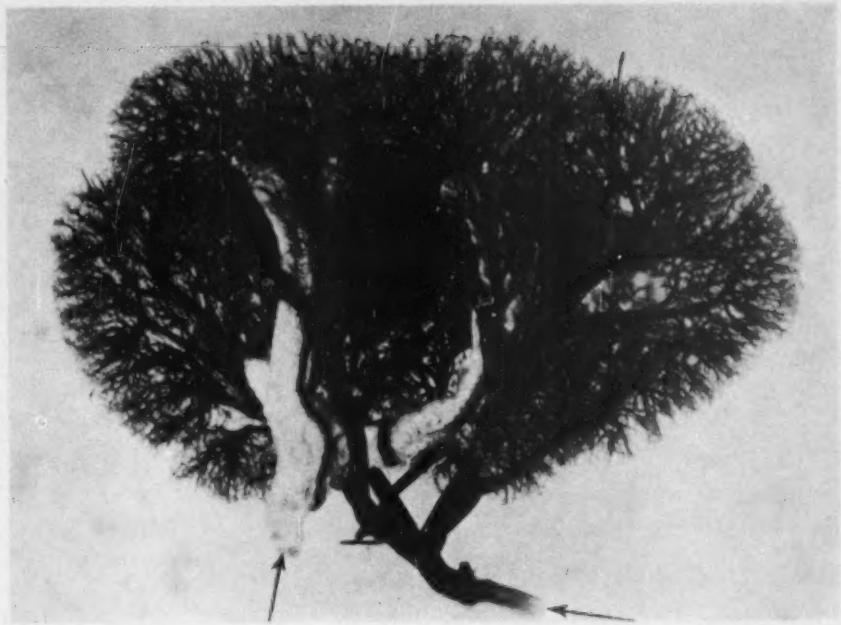

FIG. 1. A vinylite cast of the right kidney of a young Indian elephant (one-third of natural size).

SUMMARY

The distribution of the renal artery and the divisions of the ureter of an eight-year-old female elephant were similar to those of the left kidney of an older male previously described.

The arterial pattern is that which is common to most mammals.

ACKNOWLEDGEMENTS

Many thanks are expressed to R. Hood for the photographs, and to E. Roberts for his help and technical skill.

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GUZSAL, E. (1960). *Acta vet. Acad. Sci. Hung.*, **10**, 401.

BOOK REVIEWS

A NEW TEXT-BOOK OF SMALL ANIMAL ORTHOPAEDICS

Orthopaedic Surgery of the Dog and Cat. By ELLIS P. LEONARD. London: W. B. Saunders. Price 87s. 6d.

In the preface of this book Dr. Leonard writes "After twenty-five years of clinical study and observations in the field of orthopaedia the author has recorded as concisely as possible his experiences in orthopaedic surgery". This comment exemplifies one of the main features of the book, namely that it has been written in the light of vast personal experience.

In the past Dr. Leonard has not been a prolific writer and this is confirmed by looking through the list of references set out at the end of each chapter. Nevertheless his book is written authoritatively and obviously with the thought and experience gained from a long period of specialization. The author has made use of the experience of others whenever necessary but always the references are relevant and concise.

One is immediately impressed by the quality of this book. The general layout is excellent and the quality of the illustrations and photographs is outstanding. Padding and superfluous illustration have been avoided, which makes the style concise and a pleasure to read for there is a flow and unity about the book which is a rarity in veterinary text-books.

The book is divided into four parts: General considerations; Fractures; Luxations; and other orthopaedic diseases.

The first two chapters on cartilage, bone formation and bone circulation, healing and non-union make an essential introduction to the subject and give a clear understanding of the principles which underlie successful orthopaedic surgery.

The preparation for surgery, materials, patient and surgeon is clearly outlined and the need for asepsis is emphasized. Although the man in practice can seldom aspire to such high standards for reasons of economy and time the whole procedure of preparation is so well described that it forms a goal at which the serious-minded surgeon should aim. Many who read this book will be encouraged to attempt techniques which go beyond their established procedures, but the enthusiastic surgeon would do well to consider the advice given in Chapter 3 of this book and decide whether his preparation and facilities are compatible with the prolonged exposure which some of the techniques involve, for the greatest obstacle to successful orthopaedic surgery is sepsis.

Although some of the appliances described in the book are either impossible or difficult to obtain in Britain, the author gives an excellent description of some of the simpler but nevertheless successful methods of splinting and bandaging, materials for which are readily available.

The section dealing with pinning and plating will prove of particular interest to a large proportion of readers. For those who aspire to greater things the description and illustration of more elaborate appliances for immobilization will prove valuable.

Part II of the book commences with an interesting classification of fractures followed by a detailed description of fractures as they involve each bone or group of bones. It is pleasing to note that when necessary an adequate consideration of pathology and in particular the all-important aspect of blood supply and circulation is considered in relation to each fracture. Clinical signs, diagnosis and prognosis are briefly discussed but liberal space is devoted to treatment and all are related back to materials and methods described in Part I.

Fractures involving the skull, spine and pelvis are dealt with in Chapter 7, which will prove to be invaluable to the man in practice who may not meet fractures of skull and spine very frequently and is often at a loss as to prognosis and treatment.

In Part III (luxations) many elaborate methods are described for dealing with coxafemural luxation. One questions the practicability of some of these methods as they involve restraint, which one assumes is not tolerated by the patient very easily. The method which the reviewer favours, which is the one described by Dr. Vita for the treatment of this condition, is unfortunately not illustrated. This is regretted for the method deserves greater explanation.

Again, although the Stader method of correcting luxation of the patella is described this relates only to the traumatic injury. In view of the great increase in the congenital abnormality in many breeds today it is unfortunate that there is no description of surgical techniques aimed at the correction of this condition although these have been described in veterinary literature.

A chapter on intervertebral disc luxation is very well illustrated and describes an operation which will be new to many readers.

The book ends with a discourse on hip dysplasia and hypertrophic pulmonary osteoarthropathy. The description of the latter reflects the need for considerably more work on this complex condition.

This book is outstanding in many respects and meticulous care and thought have clearly gone into its production. It fills an obvious gap in veterinary literature and will be appreciated by students, teachers and practitioners alike. The author is to be congratulated on this masterly production.

W. B. SINGLETON

THE ANATOMY OF SYNOVIAL JOINTS

Synovial Joints. By C. H. BARNETT, D. V. DAVIES & M. A. MACCONAILL. London: Longmans Green. Price 50s.

The four major subdivisions that constitute this book are concerned respectively with the components, the biology, the mechanics and the function of joints. In the first section the macroscopic and microscopic make-up of each joint component is described in some detail and reference is also made to the chemical composition of these tissues. The blood and nerve supply and lymph drainage are described in the second section. Also included here are views on the metabolism and nutrition of joints, their pre- and post-natal development and the degenerative changes to which they are prone. The third section deals with the classification of synovial joints, joint movements and the mechanical efficiency of these structures. In the final section, joints are considered in

relation to the skeleton as a whole; joint mobility and the co-ordination of joints are also discussed.

The work is well illustrated and includes an extensive bibliography.

Despite a voluminous literature on most aspects of articular anatomy and function, there has long been a need for a book that provides information and views on a wide range of articular problems in a composite form. "Synovial Joints" is such a book and is to be thoroughly recommended to the graduate with an interest in any facet of joint study, whether in man or animals.

L. C. VAUGHAN

THE ILLUSTRATION OF SCIENTIFIC WORK

Art and the Scientist. By GEOFFREY LAPAGE. pp. 115, 181 references, 36 plates. Bristol: Wright and Sons Ltd. Price 42s.

Dr. Lapage proves that retirement from an active research life in parasitology can be but a phase passing into some equally profitable realm of productivity. All it requires is energy, imagination and the ability to write lucidly. The last book of his which I reviewed was in essence a special kind of history of medicine. The present effort is a different kind of history. It has enabled him to demonstrate the results of wide and prolific reading and profound contemplation. The book is well written and produced, has an excellent series of plates, some of them beautiful creations, and could be read with profit by any intelligent person in any mode of life—if he thinks about art and the scientist at all. A pen and ink drawing by Cajal of the cat's cerebral cortex makes quite an attractive cover, and makes one wonder why more use is not made of microscopic stained structures as patterns for paper and cloth.

The purpose of the book was to discuss illustrations by men of science seeking to characterize their own work, or that of other scientists. It is not a history of the art of illustration. It deals chiefly with illustrations on botany, zoology and human and comparative anatomy, for it has been these sciences which have used mostly graphic illustrations in the past, and long before the day of present photographic excellence. Pathology far outstrips them all now and I would have liked to see some comments on present-day illustration of pathology, for there is still a great deal of art in both black and white and colour photomicrography. Quality of the latter in the myriad of journals on pathology varies from the abominably useless to the superbly excellent.

The author then goes on to consider the characteristic qualities of illustrations which belong both to science and art and what goes on in the minds of the artists and scientists respectively. He debates the kind of illustrations there are, which can be purely descriptive, or interpretative or imaginative. In the first class, the aim is to depict as accurately as possible what was seen. In the second group, it is permissible to leave out certain details which might obscure the main idea in the scientist's mind and which can only be undertaken by the scientists concerned, and be understood by readers with the requisite knowledge and experience.

There can be no doubt that the developments in different sciences were

dependent on the development of art and crafts attached to production of illustrations. We need only think about the classic developments in anatomy which would have been impossible without art.

A chapter follows which discusses the origin of some classical illustrations, and for veterinary readers we can refer to the questionable origin of the illustrations in Ruini's *Anatomia del Cavallo*. It has been suggested that Leonardo da Vinci was responsible. They are of the same high calibre as those in Vesalius' *Fabrica Anatomica* (the artist being Kalkar).

The influence of the skill of the engravers who employed wood, metal or stone was obviously immense, and many scientists of the old days were themselves trained in this craft. Names of many scientists are trotted before us, most of whom in the "medical" sciences should be known to the veterinarian as well as the medical worker, for many of these men created landmarks in the history of anatomy and pathology in Britain. We read, for example, of Sir Charles and James Bell of Edinburgh, who worked on the nervous system, Stubbs and his work on the anatomy of the horse, and Carswell who produced the first treatise with illustrations on the pathology of man (and incidentally produced the earliest coloured illustration of tuberculous mastitis in the cow—reproduced by John Francis in his book on Bovine Tuberculosis).

Many examples from other fields such as botany, protozoology, zoology, and bird life are discussed at length.

Running throughout there is a philosophical vein and in particular in Section VII on the creative process in science and art, and how scientists have reached, sought and made their discoveries. There might be a good deal of debate here, and some of the examples are not quite happy ones. For example when the necessity for possessing a prepared mind to make discoveries is being discussed, the example of Sir Alexander Fleming and his original observation on the effect of mould on bacterial growth is quoted. However, it seems to the reviewer that a prepared mind is not nearly enough, for what Fleming lacked was vision and foresight to bring his discovery to its full fruition with the use of penicillin as the most powerful chemotherapeutic agent discovered until then. The man who had the prepared mind plus the vision was Florey who with his co-workers in Oxford could see possibilities in an obscure observation which had been made some 15 years earlier and lain dormant ever since. I cannot help in this connection but remember what Ehrlich was once reputed to have said about the necessary qualities for research workers—*Geld, Geduld, Glück* and *Gelegenheit*, which is pretty true if you just think for a minute. You certainly are not going to get very far if any one of these is missing.

I enjoyed the book, which stimulated thought, and I am sure many readers will do likewise. J. R. M. Innes

"BONES"

Aide-mémoire d'ostéologie comparée des animaux domestiques. By C. Bressou. Paris: Vigot Frères.

The title of this work clearly indicates its content and its purpose. The student desiring to revise his "bones" will find here a lucid exposition of the comparative

features and he may safely assume the recommendation of his French colleagues from the appearance of this second edition.

The teacher encountering the familiar facts, baldly arranged without benefit of padding, may suffer a twinge or two of conscience at this reminder of the students' burden and may be tempted to ask himself if much of this load could not be jettisoned without great loss.

K. M. Dyce

TOPOGRAPHICAL ANATOMY

Atlas der topographischen Anatomie der Haustiere. Vol. I. By Peter Popesko. Jena: Gustav Fischer.

In the introduction to this volume, Dr. Popesko announces his intention of producing a complete atlas of the regional anatomy of the domestic animals. This first part is concerned with the head and neck and consists of 200 plates with accompanying keys. The bulk of these depict the anatomy of the horse and ox, but the dog, pig and small ruminants are also adequately treated and a few plates are devoted to the cat and hare.

The need for a complete atlas of veterinary anatomy has long been obvious and all interested in the subject will be conscious of their indebtedness to Dr. Popesko. It may therefore be ungracious to point out that the standard of draughtsmanship is not high and that the plates do not compare in artistry with those in other works, such as that by Ellenberger, Baum and Dietrich, with which the reader may be familiar; also the general level of production falls far short of the sumptuous standards established by Schmaltz. Too much should not be made of these deficiencies: they do not affect the usefulness of this undertaking and must be considered in relation to the relatively modest price set on the present volume. This ensures that the atlas will be available to students and can be exposed to the hazards of the dissection room without too many qualms.

It may be useful to mention that language will present no problem since the keys are bilingual, the German vernacular names being supplemented by the familiar Latin terms.

The completion of this work will be eagerly awaited. Possibly in later parts the author will consider devoting rather more space to those aspects of anatomy that possess an obvious clinical importance: in the present part one would willingly have sacrificed some of the illustrations of cross-sections for a more ample treatment of the sinuses and larynx.

K. M. Dyce

CORRESPONDENCE

AFRICAN PIG DISEASE

Sir,

It has been recognized for at least twenty years that the disease first described by Montgomery[1] as "East African swine fever" is antigenically distinct from swine fever. Walker[2] pointed out that during the period 1910–21 when Montgomery was investigating it, swine fever was not present in East Africa.

That the name is still used in everyday conversation in East Africa is no justification for prolonging the myth. However, as long as the disease was confined to Africa little harm was done. But with its introduction to Europe and the subsequent heavy losses the situation has changed. The disease is no longer of interest only to Africa, a fact which Mr. Beaton apparently has not appreciated. It has become an important world-wide problem. The possibility of its dissemination by infected pig products from ships and planes makes it a very real menace for every country with an established pig industry. To persist in referring to this major disease as a type of swine fever would, now that it has appeared in areas in which swine fever is endemic, cause endless confusion in the future. One should be forewarned by the history of Newcastle disease, and the ease and rapidity with which it spread throughout the world. Mr. Beaton's suggestion that the name should not be changed, because there are other diseases of pigs in Africa, some as yet unrecognized, does not merit serious consideration. What about swine fever? Are there not other diseases of pigs in countries in which swine fever is endemic? The name "African pig disease" was taken from *The Times* and it was merely courtesy to acknowledge the source. The charge of journalese carries little weight. It would be equally valid to claim that "African horse sickness" was journalese because there are other diseases of horses in Africa; or cattle plague or a host of other diseases. The name "African pig disease" is of course not perfect, but the all-important point is that it does at least dissociate the disease from swine fever.

Yours faithfully,

T. M. Doyle

Central Veterinary Laboratory, Weybridge.

[1] Montgomery, R. E. (1921). *J. comp. Path.*, **34**, 159, 243.
[2] Walker, J. (1933). Thesis, Vet. Faculty, Z rich.

AFRICAN SWINE FEVER

Sir,

While not wishing to pursue the argument on revised nomenclature of the above disease, there are one or two corrections necessary to Doyle's letter.

Firstly, the name used in Africa is African swine fever and not East African swine fever and secondly, we in Africa need no reminder from the U.K. of the importance of this disease to Europe and have consistently drawn the attention of Europe to its potential danger.

I would merely add, that apart from a little stimulating discussion on the matter, I maintain that it is inappropriate to attempt to make any decision on a change of name prior to the recommendations of the body appointed specifically for study of the nomenclature of diseases of animals. One misnomer does not justify replacement by a second misnomer and we have lived for a long time with the classical example of hog cholera.

Yours faithfully,

W. G. Beaton

Interafrican Bureau for Animal Health (C.C.T.A.),
Muguga, P.O. Kikuyu, Kenya.

NEWS

INTERNATIONAL CONFERENCE ON CATTLE DISEASES

The German Veterinary Medical Association at its meeting in Hanover in 1960 decided to hold a series of international conferences on cattle diseases. The next meeting, which will be held on 17th, 18th and 19th May, 1962, will be at the Veterinary School in Vienna and it will be concerned with special pathology, diagnosis and therapy of cattle diseases. Contributions should be sent to Professor K. Diernhofer, Rinderklinik der Tierarztliche Hochschule, Vienna III, Linke Bahngasse 11, before 31st January, 1962.

SYMPOSIUM ON THE CONSERVATION OF NATURE AND NATURAL RESOURCES IN MODERN AFRICAN STATES IN COLLABORATION WITH U.N.E.S.C.O. AND F.A.O.

This Symposium was held at Arusha, Tanganyika from the 5th–12th September, 1961, under the joint sponsorship of The Commission for Technical Co-operation in Africa South of the Sahara and the International Union for the Conservation of Nature.

During the week-end, in the middle of the Conference, two-day bus tours were arranged and fully supported for visits to famous National Game Parks and Nature Reserves, such as Amboseli in Kenya and the Ngorongoro Crater in Tanganyika. The attendance at the Conference was large—over 160 delegates from 27 countries in Africa and Europe, with representation from a great many societies for the protection of nature. Some 65 papers were presented but only a proportion could be discussed in any detail.

Delegates from African countries stressed their interest in the preservation of wildlife not only from the economic point of view such as tourism, but for the purpose of education of their rising generations. It was agreed in general that where cropping of surplus game animals for protein for human consumption could provide a revenue, it should be investigated with a view to providing additional funds for wildlife management.

The relationship of wildlife to disease in domestic animals was referred to and the plea was made that veterinary advice should be sought before any new wildlife conservation scheme should be entered upon. Training in Africa on the principles of conservation and management of wildlife, as a post-graduate diploma course at University level was considered to be an essential provision, to train future game wardens and foster an interest in this field as a career.

The Meeting received with acclaim the following statement signed by the Prime Minister, Mr. Nyerere, the Minister for Lands and Survey and the Chairman of the meeting, Chief Fundikera:

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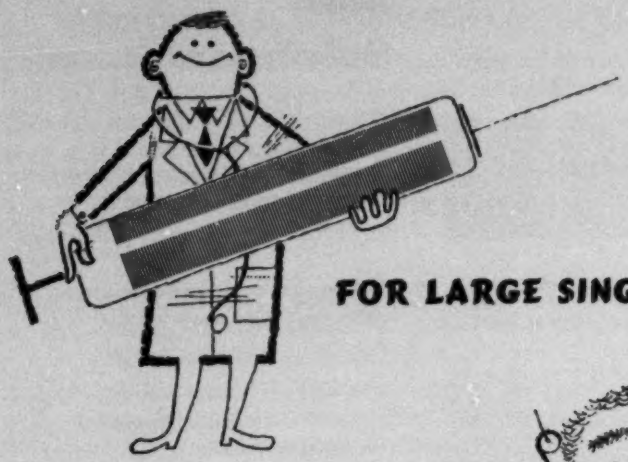

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SHEEP RECORDING AND PROGENY TESTING

In its Report on "The Sheep Industry in Britain, 1958", the National Resources (Technical) Committee stressed that very little was being done by those in the sheep industry to take advantage of the techniques of recording and progeny testing, and that the industry was in fact dependent on pedigree breeders whose objectives and methods were in some respects out of date. The Committee accordingly recommended that more attention should be given to the commercial performances of ewes and rams and that to this end recording and progeny testing should be encouraged.

The Committee on Sheep Recording and Progeny Testing was subsequently appointed in May, 1959, by the Agricultural Improvement Councils of England and Wales and of Scotland, with the support of the National Sheep Breeders' Association, to review the present position in regard to sheep recording and progeny testing and to recommend what further developments were desirable.

During the last 20 years the estimated gross output of British agriculture has, on a fixed price basis, risen by more than one half. Sheep numbers fell and advanced during this period and although they are now contributing relatively a little less to total agricultural output than before the war they must be subject to the general intensification of farming. On lowland farms in particular the improved pastures, heavy fertilizer applications and better methods of controlling disease have changed substantially the environments in which sheep are produced. These developments in husbandry, together with changes in market requirements and rising costs, call for a review of our methods of breeding for adaptation to the new conditions. It is against this background that the Committee has studied the practical aspects of recording and progeny testing when applied to sheep in Britain. Its conclusions and recommendations are published as "Sheep Recording and Progeny Testing".*

The various kinds of flocks found in Great Britain are classified, with a description of their functions and attributes. Some of the conclusions reached are that in lowland flocks the characters of prime economic importance are prolificacy, milking capacity, growth rate and carcase quality, together with fleece weight and quality in some flocks; and that in hill flocks the most important are hardiness, fleece weight and quality, and prolificacy. Possibilities

* Sheep Breeding and Progeny Testing, 37 pages, Price 2s. 6d. H.M.S.O.

of improving these characters through breeding and management are discussed. But it is emphasized that limitation of objectives in a flock is essential to such improvement. The benefits that individual sheep recording can bring to industry, both in commercial and stud flocks, are suggested—probably the most important being an aid to management. A further conclusion is that progeny testing, or some form of family selection, must be adopted if the more weakly heritable economic characters of sheep are to be improved. Considerations of the best breeding methods for specific objectives are indicated for the guidance of the individual flockmaster. Specimen breeding schemes designed to achieve specific objectives are also described.

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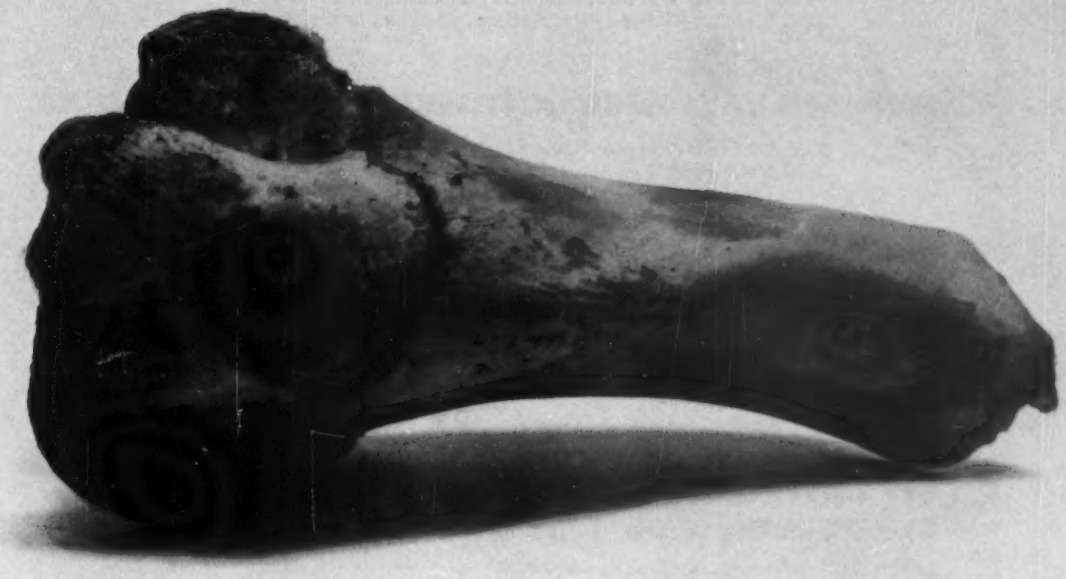

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THE BRITISH VETERINARY JOURNAL

The January issue (Vol. 118, Number 1) will contain the following papers:

J. S. S. Inglis and Mary Weipers, *Department of Animal Husbandry, University of Glasgow.*	An Assessment of the Single Intradermal Comparative Test in the Diagnosis of Bovine Tuberculosis in Cattle previously vaccinated against Johne's Disease.
Brahm Dutt and P. C. Sawhney, *Animal Nutrition Division, Indian Veterinary Research Institute, Izatnagar, United Provinces.*	Metaplasia in Experimental Vitamin A-Deficient Lamb.
Elizabeth Allan and A. E. Street, *Nutritional Research Unit, Huntingdon.*	The Effects of Incorporating Sulphadimidine-Pteridine Mixtures in the Diet of Young Chicks.
S. B. Kendall and J. W. Parfitt, *Central Veterinary Laboratory, Weybridge.*	The Chemotherapy of Fascioliasis.
J. K. L. Pearson, W. R. P. Hildebrand and T. G. McClelland, *Veterinary Research Division, Stormont, Belfast.*	Studies on Johne's Disease in Cattle in Northern Ireland: I. Incidence and Development.
J. K. L. Pearson, *Veterinary Research Division, Stormont, Belfast.*	Studies on Johne's Disease in Cattle in Northern Ireland: II. Diagnosis of Clinical Infections.

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THE BRITISH VETERINARY JOURNAL

DECEMBER 1961

LIST OF CONTENTS

The Cellular Picture of Vaginal Mucus from Normal Heifers 519
I. D. B. Newsam

Griseofulvin for *Trichophyton mentafrophytes* infection of Chinchillas 524
R. E. Ablett

Morbidity and Mortality Rates in East Coast Fever (*Theileria parva* infection) and their application to Drug Screening Procedures 529
D. W. Brocklesby, S. F. Barnett and G. R. Scott

Studies on Disinfection of Eggs and Incubators—V: The Toxicity of Formaldehyde to the Developing Embryo 532
E. G. Harry and J. A. Binstead

Serological Identification of Ox, Buffalo, Goat and Deer Flesh 540
F. C. Pinto

The Arterial Circulation of the Kidney of a Young Elephant (*Elephas indicus*) 545
T. Grahame

Book Reviews 548

Correspondence 552

News 554